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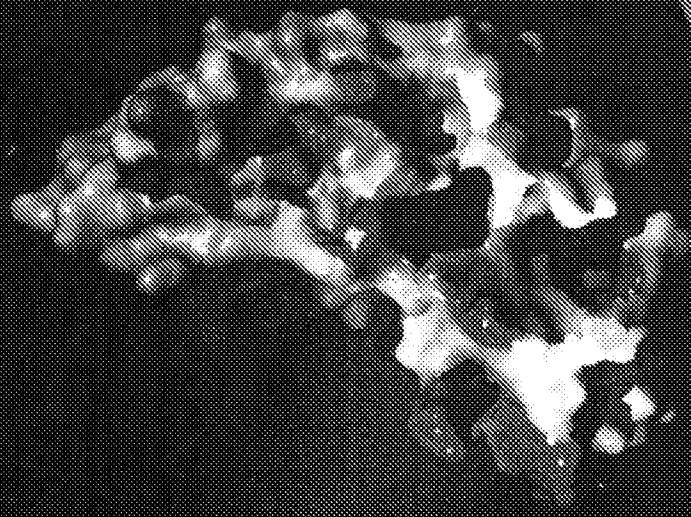

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Modulation of NF-κB Activity by Exchange of Dimers

Simona Saccani, Serafino Pantano, and Gioacchino Natoli*
Institute for Research in Biomedicine
Via Vela 6
CH6500, Bellinzona
Switzerland

Summary

Transcription factors within a family usually share the ability to recognize similar or identical consensus sites. For example, the five mammalian NF-κB/Rel proteins generate more than 12 dimers recognizing 9–11 nucleotide κB sites. Each dimer selectively regulates a few target promoters; however, several genes are redundantly induced by more than one dimer. Whether this property simply generates redundancy in target gene activation or underlies more complex regulatory mechanisms is an open issue. We show here that during dendritic cell maturation, rapidly activated dimers (e.g., p50/RelA) bound to a subset of target promoters are gradually replaced by slowly activated dimers (e.g., p52/RelB). Since the dimers have different transcriptional activity at each promoter, the dimer exchange allows fine tuning of the response over time. Further, due to the insensitivity of p52/RelB to the NF-κB inhibitors, the IκBs, dimer exchange contributes to sustained activation of selected NF-κB targets in spite of the resynthesis of IκBα.

Introduction

The nuclear factor kappa B (NF-κB) family of transcription factors (Sen and Baltimore, 1986) regulates numerous genes controlling immune response, cell growth, apoptosis, and tissue differentiation (Baldwin, 2001; Ghosh et al., 1998).

The five mammalian NF-κB/Rel proteins contain an N-terminal segment of about 300 amino acids, the Rel-homology-domain (RHD), that is responsible for DNA binding, dimerization, nuclear translocation, interaction with the IκBs, and transcriptional regulation (Siebenlist et al., 1994; Verma et al., 1995). Three family members, p65 (RelA), cRel, and RelB, contain transcriptional activation domains (TAD) at the C terminus and therefore are able to directly activate transcription. The other two members, p50 and p52, are synthesized as large precursors (p105 and p100, respectively) with an N-terminal RHD and C-terminal ankyrin repeats: ubiquitin-dependent proteasomal processing removes the C-terminal domain and releases mature p50 and p52 (Betts and Nabel, 1996; Palombella et al., 1994; Xiao et al., 2001). p50 and p52 lack a TAD and therefore form homodimers with no intrinsic ability to activate transcription. However, they form transcriptionally active heterodimers in association with p65, cRel, and RelB. Moreover, p52 can activate transcription when complexed to Bcl-3, an IκB-like molecule with coactivator functions (Siebenlist et al., 1994). Dimerization is required for NF-κB binding to DNA, and more than 12 homo- and heterodimers have been described (Thanos and Maniatis, 1995; Verma et al., 1995). Different dimers are held in the cytoplasm by interaction with specific inhibitors: dimers containing p65 or cRel associate with the IκBs (inhibitors of κB), which include IκBα, IκBβ, and IκBε (Whiteside and Israel, 1997). IκBs contain an N-terminal regulatory region that is phosphorylated in response to stimulation (Brockman et al., 1995; Brown et al., 1995; DiDonato et al., 1996) and C-terminal ankyrin repeats, which mediate association with NF-κB dimers (Huxford et al., 1998; Jacobs and Harrison, 1998). Conversely, RelB/p52 does not associate with the IκBs (Kistler et al., 1998; Lernbecher et al., 1994; Weih et al., 1994) and is retained in the cytoplasm by p100 (Dobrzanski et al., 1995; Solan et al., 2002). IκB-sequestered complexes are released by activation of the canonical NF-κB activation pathway (Karin and Ben-Neriah, 2000), which depends on the IKKβ/IKK2 subunit of the IκB-kinase (IKK) complex (DiDonato et al., 1997; Mercurio et al., 1997; Regnier et al., 1997; Woronicz et al., 1997; Zandi et al., 1997) and on its noncatalytic partner, IKKγ/NEMO (Mercurio et al., 1999; Rothwarf et al., 1998; Yamaoka et al., 1998). By phosphorylating two N-terminal serines in the IκBs, IKKβ generates a docking site for the βTrCP proteins (Spencer et al., 1999; Winston et al., 1999; Yaron et al., 1998), which polyubiquitinate the IκBs and target them for proteasomal degradation, thus liberating p65- and cRel-containing dimers. Release of p52/RelB (Senftleben et al., 2001; Xiao et al., 2001) as well as p50/RelB dimers (Muller and Siebenlist, 2003) occurs through a "noncanonical" pathway requiring the NF-κB-inducing kinase (NIK) (Xiao et al., 2001), which phosphorylates and activates IKKα (Regnier et al., 1997). In turn, IKKα phosphorylates p100, thus directing its polyubiquitination and processing (Senftleben et al., 2001). This pathway is induced in response to a subset of stimuli such as BAFF, CD40 ligand, and LTβ-R triggering (Claudio et al., 2002; Coope et al., 2002; Dejardin et al., 2002), and it has much slower activation kinetics than the canonical one. The independence of the two pathways is further indicated by the integrity of the noncanonical pathway in IKKβ−/− and IKKγ/NEMO−/− cells (Dejardin et al., 2002), as well as by the observation that BAFF selectively activates the noncanonical pathway (Claudio et al., 2002). Whether the two pathways regulate completely distinct or partially overlapping sets of genes is still an open issue. Expression of several genes (e.g., *G-CSF*, *VCAM*, and *TNFα*) is elevated without stimulation in both IκBα−/− mice (in which the major activated complex is p50/RelA) (Beg et al., 1995) and in mice lacking the C terminus of p100 but still expressing p52 (in which the major activated complex is p52/RelB) (Ishikawa et al., 1997). On the other hand, analysis of gene expression induced by LTβ-R triggering indicates that in fibroblasts, some genes are nonredundantly regulated by either of the two pathways (Dejardin et al., 2002).

An obvious requisite for redundancy is that more than

*Correspondence: gioacchino.natoli@irb.unisi.ch